CLINICAL METHODS OF ESTIMATING THE DEGREE OF ACIDOSIS IN DIABETES.*

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What are the possible ways by which the general practitioner, who has not much time at his disposal, may follow from day to day the progress of his diabetic patients as regards the condition which is termed acidosis?

First let us consider the most direct method, the estimation of the amount of acetone bodies excreted in the urine.

No one of the methods used can lay claim to absolute quantitative accuracy. This is especially true of oxybutyric acid. The method of Magnus Levy—Lind's apparatus for the preliminary extraction considerable shortens the time required for the application of this method—which is generally admitted to be the best, requires an expensive and complicated apparatus and much time, skill and experience. As regards the acetone, a variable and incalculable amount is lost in the breath. Even if these methods did give accurate results, they could not be accepted as an absolute criterion of the clinical condition of the patient. For in the first place, no fixed relationship can be established between the acetone bodies in the urine and the resulting dangers to the patient. Some diabetics without any symptoms will for months at a time excrete quantities of oxybutyric acid which are as large as those found in other cases in whom the severest symptoms of acidosis are present. Even in the same individual the resistance to the toxic action of these substances appears to vary considerably. And finally, it must always be remembered that the amount of acetone bodies in the urine is no certain guide to the quantity present in the body. It is not the amount in the urine but that part which is present in the tissues which is doing the damage, and in many cases it appears as if the onset of diabetic coma was due not so much to a sudden over-production of acetone bodies, as to a failure to get rid of them. Everyone has seen how the giving of alkali will increase the excretion of acetone bodies and will help to remove considerable amounts which would otherwise be retained, so that excretion does not by any means always tally with production.

In view of these facts and of the technical difficulties of the quantitative methods, there is obviously great need for some simple readily applied method whereby an approximate idea can be obtained as to the amount of acetone bodies, for in spite of the difficulties in the interpretation of the results, the determination of the quantity of these substances remains one of the best methods at our disposal in judging the degree of acidosis present.

Such a method has been introduced by Hart.[1] It depends on the quantitative relationship which exists between oxybutyric acid and diacetic acid. Since diacetic acid is simply an oxidation product of oxybutyric acid, an increase in oxybutyric acid is usually associated with an increase in diacetic acid. When oxybutyric acid is given to a diabetic, the amount of diacetic acid rises. In any severe acidosis, the amount of acetone is negligible compared with the quantity of the other two bodies, in mild acidosis only small amounts of diacetic acid and some acetone are present, and in the mildest of all, acetone alone is found. Of the three acetone bodies, diacetic acid is the only one of which a rapid approximate determination can be made, and from this in most cases, a fairly accurate idea of the total amount of the three bodies present can be obtained. The method is a colorometric one. The depth of the red color produced by ferric chloride in a solution of ethy-aceto-acetate of known strength is compared with the color produced in the urine. We have found that the reading may be very accurately and rapidly made by using the colorimeter recommended by Rowentree and Gerahty for use with the phenolsulphonephthalein test. But the reading may also be made simply by diluting the urine in a graduate until the standard color is attained.

Another test for diacetic acid has been lately given by v. Onderjowich[2] which can also be adapted for use as a rough quantitative method. Methylene blue is decolorized by iodine, but if diacetic acid is present in the urine the dye is protected because the iodine is all taken up by the diacetic acid with which it forms a colorless addition product. The amount of iodine required to decolorize the methylene blue depends on the quantity of diacetic acid present.

Both these methods take only a few minutes. We have made a large number of observations with them and find that in cases of moderate acidosis, such as are seen for instance when a strict carbohydrate-free diet is instituted, they appear to give a fairly accurate idea of the grade of acidosis as far as can be judged from the fact that they follow the variations of ammonia excretion. But in a case of diabetic coma the relationship seemed to fail, for the quantity of oxybutyric acid present was much larger than the total acetone bodies as given by these methods. The quantitative estimations made by Hart with Schaffer's method gave results which agreed quite closely with the readings made with his own method, but *they* were cases of moderate acidosis and it is certain that such a close correspondence would not have been found at least in all cases of severe acidosis. In the last resort, we know that diabetic acidosis is due to a failure to oxidize fatty acids and it appears that in the last stages of some cases of severe acidosis this failure in oxidation may go so far as to bring about a complete absence of the conversion of oxybutyric acid to diacetic acid. Stadelmann reported three cases of typical diabetic coma in whose urine he was able to show the presence of large amounts of oxybutyric acid and yet was unable to obtain a positive reaction for diacetic acid. And when Magnus Levy's figures[3] of the amounts of acetone plus diacetic acid are compared with the quantities of oxybutyric acid found in cases of diabetic coma, it is seen that there is absolutely no

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relationship between them whatever. Here, therefore, these methods may fail us and it must always be remembered that the diagnosis of a condition of coma due to acidosis cannot be excluded because of the presence of only a small amount of diacetic acid. However they are, because of their simplicity and especially when taken in conjunction with other estimations, extremely useful clinical methods.

The other means we possess of determining the degree of acidosis are all indirect and are based on a measurement of the effects of the acids on the factors concerned in the maintenance of the neutrality equilibrium of the body. So long as life continues, no appreciable variation from neutrality ever occurs. Such an alteration in reaction is incomparable with the physical conditions and ferment actions essential to the extraordinarily complex interrelated chemical reactions on which life depends. This constant state of neutrality, in spite of the production of very large amounts of acid in the body, depends on two main factors, the capacity to excrete more acid than basic radicles, and the power of neutralization which the tissues and fluids of the body possess.

The excretion of acid in excess of base takes place through two main channels, the kidneys and the lungs. The loss of carbon dioxide in the breath is a purely acid excretion. The urine in cases of acidosis has an acid reaction though the blood from which it is secreted is neutral. The kidneys, therefore, in some way or other, remove an acid from a neutral fluid. In cases of marked acidosis one might expect to find a very high degree of acidity in the urine. We worked this point out in a series of cases but were unable to find any close correspondence between the degree of acidity of the urine and the grade of acidosis as judged by other methods. With the development of acidosis, there was indeed in general a moderate increase in acidity, but even in cases of diabetic coma, the change was not pronounced. There appears to be a very definite limitation of the power of the kidneys to excrete acids. These results were obtained with Folin's method,[4] and at the same time a parallel series of estimations were made by the method of Adler and Blake.[5] They use a standard mixture of acid and basic phosphates of the same H ion concentration as the blood. With resolic acid this gives a certain tint of yellow. The urine after removal of calcium and dilution is titrated with sodium hydrate until the same color is produced. This is an indirect method of expressing the difference in the H ion concentration of the blood and of the urine, and shows the amount of acid in excess of basic radicles which have been excreted by the kidneys. The results of this method were found to run parallel with those of Folin's. The increase in acidity was not proportional to increase in the grade of acidosis as judged by the ammonia output, and the conclusion must be drawn that variations in the acidity of the urine do not furnish any sure guide as to the degree of acidosis. Direct estimations of the H ion concentration of the urine would give the same result. V. Shramlik has shown that if the calcium is removed from the urine as in Folin's method, the curves by the two methods vary together.

With regard to the excretions of carbon dioxide, the other means which the body takes of ridding itself of acid, the outlook is more promising. What little work has so far been done goes to show that this may turn out to be one of the most accurate methods. The carbon dioxide in the breath is proportional to that in the blood, and the method has in this the great advantage that it is not influenced by variations in excretion as are all urinary estimations. It appears that with increasing acidosis the alkali available for the transport of carbon dioxide as sodium bicarbonate diminishes, and the excretion of carbon dioxide falls, so that this factor in maintaining neutrality fails altogether. Staub,[6] in an extensive series of estimations which have been recently published, found that there was not always any parallelism between an increase of the amount of acetone bodies in the urine and decrease of carbon dioxide in the breath. He concludes that the carbon dioxide method is the safer guide since it shows the amount retained rather than the amount excreted. Still it is not yet by any means certain that the diminution of CO_2 which undoubtedly occurs in advanced acidosis is due to a diminution of the alkali available for the absorption of CO_2 for Beddard, Pembrey and Spriggs found that the blood of patients which showed a low carbon dioxide content was capable in vitro of taking up the normal quantity. And if the theoretical foundation of the method is insecure, it is also true that the practical application has clinical limitations, for it requires some practice and skill and the intelligent co-operation of the patient.

Another series of methods is based on the other main factor in preventing any change of reaction in the body, the power of neutralization. This power depends on the property possessed by mixtures of acid and basic phosphates, of sodium bicarbonate and carbon dioxide and of most proteins, of preventing the setting free of H ions in a solution. This function is performed so well that, as Benedict[8] has shown, even in cases of extreme acidosis there is scarcely any appreciable increase in the H ion concentration of the blood and in some cases none at all.

Nevertheless, there are limitations to this capacity for neutralization for when much acid is excreted a considerable amount of fixed alkali goes with it and if there were no other source of alkali, the body would become depleted of its sodium, potassium and magnesium. It is here that another factor comes into play, the potential neutralizing power of ammonia. Under normal conditions all but a small part of the ammonia unites with carbon dioxide and is synthesised to urea. But in the presence of free acids, the ammonia neutralizes them and they are excreted as ammonium salts in the urine. An increase in the ammonia of the urine may therefore be taken as an indication of an inability of the body to cope with the neutralization of acids in the ordinary

way, for it is a reserve store of alkali called into action only as need requires. Ammonia estimations are the best clinical means we have at present of gauging the degree of acidosis. Several simple clinical methods have been recommended, but they are not very accurate and Folin's method of making the urine strongly alkaline and carrying the ammonia over into a known quantity of acid by means of an air current is so simple and at the same time so exact that it is much the best method to use. Of course, the ammonia fails to give any help at all in those cases where alkali has already been given as medication, for the diminution in ammonia bears no definite relation to the amount of alkali given. This is probably because in severe acidosis the tissues become depleted of alkali and when it is given they absorb and hold it. Indeed, this capacity to retain alkali has been recommended by Sellards [9] as a rough way of determining the degree of acidosis, and in some cases this may be of practical value. He gives increasing doses of alkali until the urine becomes alkaline. Blum [10] states that if the urine can be kept alkaline with 50 grams of sodium bicarbonate a day, there is no immediate danger of diabetic coma.

The main defect of ammonia as a gauge of acidosis, however, lies in the fact that it is only one of the neutralizing substances, and it is found that the part played by the fixed alkalies in neutralizing oxybutyric acid even in marked acidosis may be considerable and is also very variable. So that a fall in ammonia on one day may be due to an increase in the fixed alkali while an increase on another day may be due to a decrease in fixed alkali. This may be seen on examining Stadelmann's [11] figures who made determination of all basic radicles in the urine. Such estimations are, of course, quite impossible in routine work.

Adler and Blake [5] have advanced the idea of what they term the fixed alkali retention of the body as a guide. The ammonia is a means whereby the fixed alkali of the body is spared. The difference between the acidity of the urine and of the blood also represents so much alkali saved to the body. By adding the two together, they believe a better idea is gained of the strain put on the factors concerned in maintaining neutrality. The results we obtained with this method showed variations corresponding with the other evidences of acidosis, but the concordance was due almost entirely to the ammonia, so that the method does not appear to have much advantage over the ammonia estimations alone.

In conclusion, I should like to mention one other method [12] which is extremely simple and may sometimes prove of use in the diagnosis of diabetic coma. A drop of the patient's serum is applied to some filter paper soaked in resolic acid. A red color is produced. If, however, there is a even slight increase in H ion concentration of the serum, a brownish color results. We tried this on about forty sera and always found the same reaction. The only case in which a positive reaction was obtained was a case of typical diabetic coma. A case of bronzed diabetes, who was admitted in a comatose condition, did not give a positive reaction and a study of the ammonia excretion led to the conclusion that the coma in this case was not due to acidosis. In view of the dangers associated with the intravenous injection of large amounts of alkali, it is very necessary to be sure that any case of diabetes seen in an unconscious condition is one of true diabetic coma before instituting treatment of this description.

To sum up then, while we have to admit that at the present time we have no sure and certain guide in any of the methods I have mentioned, yet in dealing with any particular case, we shall by a combination of these methods and by a close study of the clinical condition, be able to gather sufficient indication of the development of a dangerous degree of acidosis to enable us to commence energetic treatment before it is too late.

References:

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Discussion.

Dr. R. L. Wilbur, San Francisco: Diabetic coma is such a startling condition and its prognosis is usually so hopeless, that if we can protect our diabetic patients by one means or another from the culminating feature of acidosis, it is most desirable to do so, and we should make use of any known method that is of value in watching the progress of our patients. I think that Dr. Addis has said that the estimation of ammonia is one of the most valuable methods of giving us an understanding of the real situation and of the strain placed upon the body in overcoming the abnormal amounts of acids formed. Folin's method is comparatively simple. It is worthy of note that Dr. Addis' observations indicate that tests for diacetic acid are not always reliable guides as to the amount of acidosis. The great disappointment we frequently meet in the alkaline treatment of severe acidosis shows that we must begin alkaline and diatetic treatment early. I have been much disappointed with the intravenous administration of carbonate of soda solutions in diabetic coma and am impressed with the fact that other factors than that of acidosis are present, particularly evident seems to be the marked disturbance of the hepatic function. It is also probable, judging from experiments on rabbits, performed by me some years ago, that the neutral salts of oxybutyric acid, after neutralization, are likewise toxic.

It is only by such careful clinical studies as these of Dr. Addis that one can obtain a clear conception of what goes on in the metabolism of the diabetic, and until we are able to gather together a large amount of data along this line it will be impossible for us to handle these abnormal conditions in a satisfactory manner from the therapeutic standpoint.